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Europ. J. clin. Invest. 4, 65—69 (1974)

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Influence of Perchlorate on the Secretion of Non-Thyroxine Iodine by the Normal Human Thyroid Gland

H. Bürgi, M. Benguerel, J. Knopp, H. Kohler, and H. Studer

Medizinische Universitätsklinik, Inselspital, Bern, Switzerland,
and Institute for Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Czechoslovakia

Received: July 31, 1973, and in revised form: October 31, 1973

Abstract. Several authors have postulated that endogenous iodide produced by the deiodination of iodotyrosines in the thyroid feeds into a different thyroidal iodide compartment than transported iodide which enters the gland from outside. One argument for the existence of two separate iodide compartments is the observation that under certain experimental conditions perchlorate completely discharges transported iodide from the thyroid, while it has no such effect on endogenous iodide. This latter observation however has not been confirmed by all studies and remained controversial. — We therefore reinvestigated the effect of perchlorate on the secretion of endogenous iodide by a new, sensitive method. Five normal volunteers received tracer amounts of iodide-^{125}I p.o. and 11 days later thyroxine-^{131}I I.V. Two days later the following serial measurements were started: serum protein-bound labelled iodine (PB^{125}I, PB^{131}I), serum total thyroxine and urinary excretion of ^{125}I, ^{127}I and ^{131}I. — In the control period the non-thyroxine iodine secretion calculated from the above measurements was 40 μg/day. Under perchlorate 200 mg three times daily this value rose significantly to 66 μg/day. Non-thyroxine iodine comprises the secreted triiodothyronine plus the secreted endogenous iodide. Assuming that the former value remained constant, our data show that perchlorate indeed discharges part, though not all, of the endogenous iodide. These data do not rule out a second iodide compartment, but they are also compatible with a simple one compartment model.

Key words: Perchlorate, thyroid, endogenous iodide, non-thyroxine iodine.

It is an accepted fact that the thyroid gland possesses two sources of inorganic iodide [1]. The first is the iodide which is accumulated from outside the cell by an active transport mechanism, the so called "transported iodide". The second sources are the iodothyrosines, which are derived by hydrolysis from thyroglobulin and rapidly deiodinated within the gland to yield the so called "endogenous iodide".

While there is general agreement that both iodide sources are used within the thyroid gland for organic iodination and therefore for hormone biosynthesis, it is still very controversial whether they actually feed into the same intrathyroidal iodide compartment [2, 3].

An important argument for the existence of two iodide compartments was based on the effect of perchlorate. This drug led to a rapid depletion of transported radioiodide in the rat, but seemed to have no effect on endogenous iodide [2, 3]. In line with this view are the observations in man that perchlorate, though preventing the active transport of iodide from outside into the gland, does not appear to discharge internal iodide [4–6].

These observations however have not remained unchallenged. In the dog, perchlorate [7–9] or thiocyanate [10] led to a massive discharge of endogenous iodide. Greer *et al.* [11] have confirmed the discharge of endogenous iodide by perchlorate in the rat, and they thought that the evidence for a second iodide pool was artifactual, a view also expressed by Wollman [12].

We have therefore considered it worthwhile to reinvestigate this controversial perchlorate effect on endogenous iodide in normal men by a very sensitive new double isotope method. The results show that perchlorate indeed leads to a small but significant increase of non-thyroxine iodine secretion which is most probably due to a discharge of endogenous iodide.

Methods

Five healthy persons served as volunteers for the investigation. The experimental set-up was adapted from the method of Wartofsky *et al.* [13–15] as detailed in a previous publication [16] and using the same material.

Experimental Protocol

Day 0: Oral intake of 80 μCi Na ^{125}I. Day 11: Intravenous injection of 30 μCi L-thyroxine-^{131}I. After a further two days allowed for equilibration of the thyroxine-^{131}I blood samples were drawn daily or at two day intervals from an arm vein. Urine samples were collected overnight during an exactly recorded time varying from 6 to 9 h. Whenever the drug regimen was changed four 6 h collections were made during one day. Time and dosage of drugs are recorded in the result section.

Measurements and Calculations

^{127}I in urine was measured by the method of Stolc and Knopp [17] in one 48 h urine sample of each subject at the beginning and in all overnight urines thereafter. The daily ^{127}I excretion in the control period was 62 μg when measured in the initial 48 h urine specimen and 59 μg when calculated from three consecutive

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overnight specimens. The agreement of both values indicates that overnight urines are valid substitutes for 24 h collections. Since the subjects were eating their usual non-standardized diet, their urinary iodine excretion as expected varied from day to day [18]. To obtain more accurate values the experiment was repeated exactly in subjects No. 1 and 4, omitting the isotopes and measuring only the ^{127}I in the urine and the mean values of the two experiments were used for these two persons.

Calculations were done as described in a previous publication [16] with the exception that the fractional thyroidal uptake of radioiodide was directly obtained from the cumulative urinary isotope excretion during the 48 h following intake of ^{125}I.

Differences in mean values were statistically evaluated with student's *t*-test [19].

Results

Table 1 gives details of the experimental subjects together with the results of routine thyroid function tests.

Fig. 1 gives the radioactivity (^{125}I) of the serum iodide and the urinary excretion of ^{125}I and of ^{131}I. The latter is divided by the PB^{131}I of the corresponding day to correct for variations in PB^{131}I which is the source of the urinary ^{131}I. Perchlorate and later carbimazole each lead to a stepwise increase of the ^{125}I of serum which is adsorbed to the amberlite resin and mainly represents iodide[1]. The urinary excretion of ^{125}I rises exactly in parallel with the serum iodide-^{125}I. Perchlorate produces an increase of the urinary ^{131}I excretion which is identical to the one predicted on the basis of the known fractional thyroidal iodine uptake (predicted rise in ^{131}I excretion $= 40.6 \pm 3.8$%, observed rise 42.9 ± 4.9%, the excretion under perchlorate being taken as 100%). This observation, together with other reports from the literature [6] suggests that the dose

Table 1

Subject No. (No., sex)	Age (years)	Weight (kg)	Thyroidal uptake of ^{125}I (% dose at 48 h)	Initial serum thyroxine (μg/100 ml)
1, ♀	25	60	30.7	11.2
2, ♂	24	70	41.0	12.4
3, ♀	26	51	37.0	8.6
4, ♂	27	76	40.1	11.2
5, ♀	24	52	54.0	9.9
Mean			40.56	10.66
Standard error			3.81	0.64

1 Mono- and Diiodotyrosine would also be adsorbed.

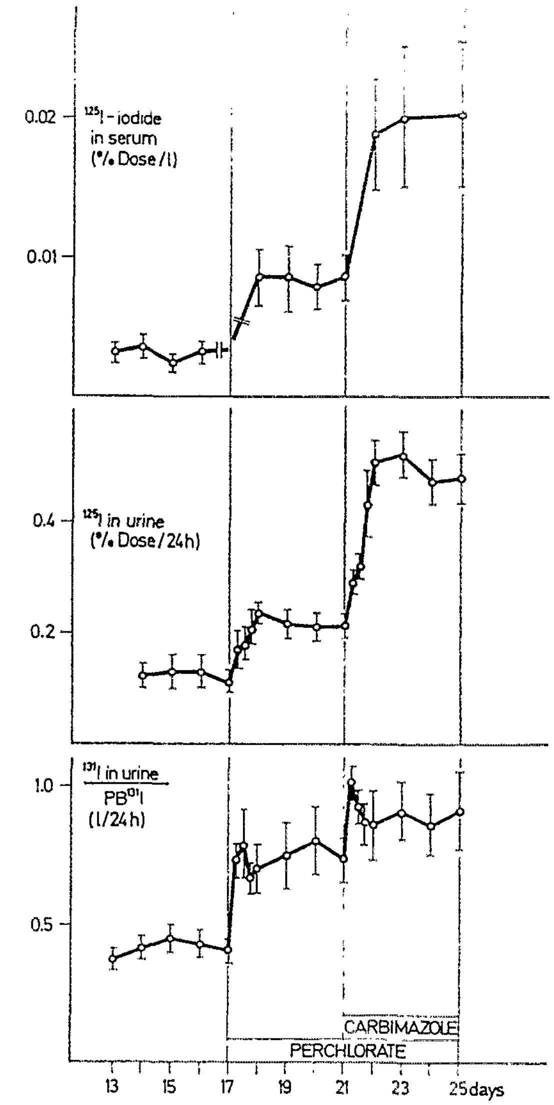


Fig. 1. Radioactivity (^{125}I) of serum iodide (top), urinary excretion of ^{125}I (middle) and ratio of urinary ^{131}I/serum protein-bound ^{131}I (bottom). The mean values with standard errors of all 5 subjects are given. Perchlorate was given in a dose of 200 mg three times daily from day 18 through to day 25. The carbimazole dose was 15 mg three times daily from day 22 through to day 25

of perchlorate was sufficient to completely block iodide uptake by the thyroid. Addition of carbimazole to the drug regimen produces a further slight rise of the urinary ^{131}I which must be due to the fact that during the 7 day control period after the injection of the thyroxine-^{131}I some of the radioiodine had been taken up by the thyroid.

The thyroidal secretion of non-thyroxine iodine as calculated from the above data is depicted in Fig. 2. In the control period the mean secretion was 40 ± 6 μg

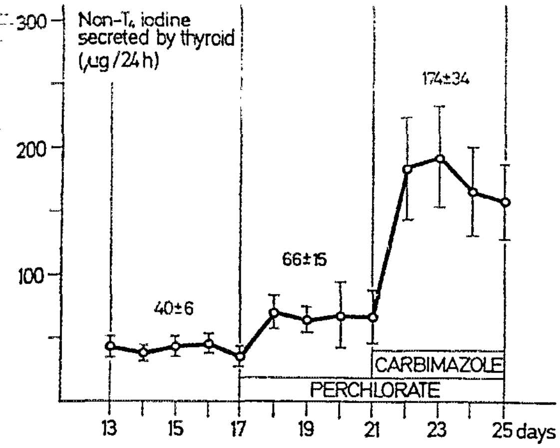


Fig. 2. Thyroidal secretion of non-thyroxine iodine as calculated from data of Fig. 1 and Fig. 2. The mean values of all 5 subjects with the standard errors are given. The abscissa and the drug treatment were the same as in Fig. 1

per 24 h (mean ± SEM). In 4 of the 5 subjects non-thyroxine iodine secretion rose significantly under perchlorate (p value <0.05 or better) and in one it decreased slightly, but not significantly. Under carbimazole plus perchlorate the secretion of non-thyroxine iodine rose further significantly in all 5 persons to a mean value 174 ± 33 µg per day. The mean values in the control period and under carbimazole are practically identical to those reported for 9 other subjects investigated under similar conditions [16].

Table 2 gives the details of the urinary excretion of ^{127}I together with a calculation of the thyroidal iodine balance. The excretion of ^{127}I rose from 60 µg in the control period to 200 µg/day in the carbimazole period (Table 2, lines b and i). An unexpected finding was that the thyroid glands of all 5 subjects were in a slightly negative iodine balance in the control period (Table 2, line f). The cause for this finding could be an error in the chemical measurement of urinary ^{127}I. We think that this is unlikely, particularly since the whole

Table 2

		Subject 1 [a]	2	3	4 [a]	5	Mean and SEM
Control period	a) Thyroidal radioiodine uptake (fraction of dose)	0.31	0.41	0.37	0.40	0.54	0.40 ± 0.03
	b) Urinary excretion of ^{127}I (µg/day)	59	62	53	74	52	60 ± 4.0
	c) Thyroidal secretion of thyroxine iodine (µg/day)	53	57	46	71	39	53 ± 5.4
	d) Thyroidal secretion of non-thyroxine iodine (µg/day)	29	24	54	54	42	40 ± 6.3
	e) Thyroidal absolute iodine uptake: $a \times (b+c+d)^{b}$ (µg/day)	44	59	57	80	72	62 ± 6.2
	f) Thyroidal iodine balance in control period: $e-(c+d)$	−38	−22	−43	−43	−9	−31 ± 6.9
Perchlorate period	g) Urinary excretion of ^{127}I (µg/day)	98	131	133	191	119	134 ± 15.4
	h) Thyroidal secretion of non-thyroxine iodine (µg/day)	60	41	120	76	34	66 ± 15.3
Carbimazole period	i) Urinary excretion of ^{127}I (µg/day)	180	153	245	269	152	200 ± 24.2
	k) Thyroidal secretion of non-thyroxine iodine (µg/day)	146	131	285	212	96	174 ± 33.5
	l) Expected rise of urinary ^{127}I from control to carbimazole period: $e+k-d$ (µg/day)	161	166	288	238	126	196 ± 29.4
	m) Observed rise of urinary ^{127}I from control to carbimazole period: $i-b$ (µg/day)	121	91	192	194	100	140 ± 22.3
	n) Observed rise of urinary ^{127}I in percent of expected rise: $100 \times m/l$ (%)	75	55	67	82	79	72 ± 4.8

[a] The ^{127}I measurements in the urine for these subjects are the mean values of two separate experiments.

[b] Assuming that iodine from endogenous sources (non-thyroxine iodine and iodine from thyroxine breakdown) is equally available to the thyroid for reutilization as exogenous iodine.

experiment was repeated in two of the subjects with excellent agreement to the first ^{127}I measurements. More likely this result must be related to findings by Dworkin [20] and Vought [21] that normal persons are in negative iodine balance most of the time if they are followed under carefully controlled metabolic ward conditions. Dworkin [20] has provided the following plausible explanation for this phenomenon: on a few days of the year a large excess of iodine is consumed, e.g. in the form of iodine-containing medications or iodine-rich food. During these days the iodine balance becames strongly positive and the thyroid gland gets "loaded" with iodine, which it slowly clears during the long periods of average iodine intake. Our subjects were instructed not to eat sea-food nor to take iodine-containing medications during the study. It is therefore not surprising that an "iodine load" with a positive balance was not observed during the 4 days of the control period.

The isotope data allow calculation of the expected rise of urinary ^{127}I from the control to the carbimazole period. By comparison of this calculated value with the value actually measured the validity of the double isotope method can be checked (Table 2, lines l and m). The data show that the double isotope method overestimates non-thyroxine iodine secretion by 28% (Table 2, line n). This systematic error is most likely due to underestimation of the specific activity ($^{125}I/^{127}I$) of thyroidal iodine, a value which cannot be measured directly in man [16]. Thus, while our ^{127}I measurements confirm the general validity of Wartofsky's double isotope method [13–15], they show at the same time that the absolute values of non-thyroxine iodine secretion it yields should be interpreted with some caution, since they may contain an appreciable error.

Discussion

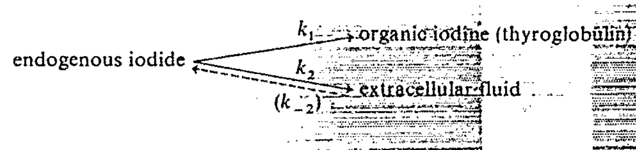
As we have outlined elsewhere [16] non-thyroxine iodine comprises between 9 to 24 μg of triiodothyronine. Quantities in excess of this value must be due to the secretion of non-hormonal iodine, which most probably represents iodide derived from the intrathyroidal deiodination of iodotyrosines.

Granted that the secretion of triiodothyronine remains constant, any increase of non-thyroxine iodine secretion must be attributed to additional secretion of endogenous iodide. Our experiments show that perchlorate indeed leads to a small but significant increase of non-thyroxine iodine secretion in 4 out of 5 subjects, and therefore, under the above premise, to an increase of the secretion of endogenous iodide of 26 μg/day. If the secretion of triiodothyronine, or that of thyroxine, had actually declined under perchlorate, then this change in endogenous iodide secretion would have been even greater. Our finding disagrees with reports by Nagataki and Ingbar [3] and by DeGroot and Bühler [6] who thought that in man perchlorate only prevented the uptake of exogenous iodide, but had no effect on the secretion of endogenous iodide. We think that the discordant results must be due to the less sensitive method used by the above investigators, who may have missed a small increase of 26 μg/day. Nicoloff [22] using a method basically similar to ours found a temporary increase of iodine release under perchlorate, lasting only about 24 h. However his method did not take into account the $PB^{131}I$ and the $PB^{125}I$ and the results cannot be fully compared. The marked rise of non-thyroxine iodine secretion and also of urinary ^{127}I excretion under carbimazole in our subjects is in good agreement with previous findings by other authors [4–6]. The results are in keeping with the view that thionamide drugs block the organification of transported as well as endogenous iodide, the latter then being secreted quantitatively.

Contrary to our finding in man perchlorate produced the secretion of *all* the endogenous iodide in the rat thyroid gland perfused *in situ* [11]. This quantitative difference may be explained by the fact that in this latter experiment the thyroids were under maximal TSH stimulation. In support of this explanation Rosenberg *et al.* [8] observed that in the dog perchlorate discharged only small amounts of endogenous iodide under basal conditions, but large quantities under TSH stimulation.

Yamada [23] has shown that perchlorate added *in vitro* at a high concentration to rat serum displaced thyroxine from albumin, the main binding protein in the rat. It is unlikely that such a displacement takes place in man where thyroxine-binding globulin is the main binding protein and where drug serum levels attained *in vivo* must be much lower. Even if such a displacement had taken place in our experiment, it would not influence our measurements of the non-thyroxine iodine secretion. The double-isotope method, thanks to the thyroxine-^{131}I injected as an internal standard, will automatically correct for any peripheral effect of drugs.

The effect of perchlorate on endogenous iodide is at first view difficult to explain. As has been pointed out by Rosenberg *et al.* [8] endogenous iodide can be disposed of either by reincorporation into thyroglobulin or by secretion, as schematically illustrated.



The fraction of endogenous iodide secreted will depend solely on the ratio of the two reaction rates k_1 and k_2. Normally k_1 is greater than k_2 and little endogenous iodide is secreted. If one takes for granted that perchlorate only blocks the influx of iodide from the extracellular fluid into the cell (a view that may be challenged), it should theoretically have no effect on the secretion of endogenous iodide. That perchlorate *does* increase the secretion of iodide could mean either a) that it increases k_2 and that secretion of iodide is not a simple diffusion process as usually accepted, or b) that one has to consider a third reaction, namely re-entry of part of the iodide just secreted into the surrounding fluid, i.e. iodide that has left the cell, but not yet left the gland [11]. We prefer this latter explanation because it fits better with the prevailing view on perchlorate action. By the same token we think that an incomplete discharge of endogenous iodide by perchlorate may not be used as evidence for a so-called second iodide compartment. Considering the above simple model it would indeed be unexpected that perchlorate discharged all the endogenous iodide. Thus admittedly our data do not rule out the existence of a second iodide compartment, but they can be explained by a simple one compartment model.

Acknowledgement. This work was supported by a grant from the Schweizerische Nationalfonds zur Förderung der wissenschaftlichen Forschung.

We want to thank Miss E. Maier for her expert technical assistance.

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Dr. med. Hans Bürgi
Medizinische Univ.-Klinik
Inselspital
CH-3010 Bern
Switzerland